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UV-Spectrophotometric Determination of Ofloxacin in Bulk and Pharmaceutical Dosage form Using Hydrotropic Solubilization Technique

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ABSTRACT

Hydrotropicsolvents may proper choice to preclude the use of organic solvents so that, a simple, accurate, novel, safe and precise method could developed for estimation of poorly water soluble drug,. Solubility of ofloxacin is increased by using 8M urea as a hydrotropic agent. Ofloxacin showed the maximum absorbance at 288 nm in method A, 284-292 nm in method B and 316nm in method C. At these wavelengths, hydrotropic agent and other tablet excipients did not show any significant interference in the spectrophotometric assay. The developed methods were found to be linear in the range of 3-15 µg/ml for method A, 1.5-7.5 µg/ml for method B&C with correlation coefficients (R) of 0.999, 0.996 and 0.992 respectively. The mean percent label claim of tablets of ofloxacin in formulation estimated by the proposed methods was found to be97.52%. The developed methods were validated according to ICH guidelines and values of accuracy, precision and other statistical parameters were found to be good accordance with the prescribe values. As hydrotropic agent was used in the proposed methods, these methods were eco-friendly and it can be used in routine quantitative analysis of drug in bulk and dosage form in industries.

Keywords: Ofloxacin; urea; AUC; Hydrotropic solubilization technique; derivative spectroscopy.

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INTRODUCTION

The term hydrotropic agent was first introduced by Neuberg (1916), to designate anionic organic salts which, at high concentrations, considerably increase the aqueous solubility of poorly soluble solutes. The hydrotropic agents are defined as non-micelle-forming substances, either liquids or solids, organic or inorganic, capable of solubilizing insoluble compounds. Hydrotropic agents consist generally of two essential parts, an anionic group and hydrophobic aromatic ring or ring system. The anionic group is obviously involved in bringing about high aqueous solubility, which is prerequisite for a hydrotropic substance. On the other hand, planarity of the hydrophobic part has been emphasized as an important factor in the mechanism of hydrotropic solubilization. Hydrotropes commonly used includes sodium benzoate, sodium acetate, sodium salicylate, nicotinamide, urea, trisodium citrate, sodium ascorbate, piperazine, caffeine, potassium citrate etc. hydrotropic agents have been observed to enhance the solubility of various substances in water. Ofloxacin was chemically (RS) 9-fluoro-2, 3dihydro-3-methyl-10-(4methl-1-piperazinyl)-7- oxo-7H-prido [1, 2, 3-de]-1, 4 benzoxazine-6-carboxylic acid. Ofloxacin belongs to class of drugscalled quinolone antibiotics. Ofloxacin is a broad spectrum antibiotic that is active against both Gram-positive and Gram-negative. It inhibition of topoisomerase enzymes, which inhibits relaxation of supercoiled DNA and promotes breakage of double stranded DNA. It is used to treat a variety of bacterial infections. Literature survey revealed that very few methods have been reported for the analysis of Ofloxacin which include UV spectroscopy[1], Reverse Phase High performance Liquid Chromatography[4], HPTLC[2] Titrimetric methods[5] and USP, BP [6, 7]. The present study illustrate development and validation of simple, economical, selective, accurate, precise spectrophotometric method for the determination of Ofloxacin by using hydrotropic solubilization technique in bulk and Pharmaceutical dosage forms and validated as per ICHguidelines.

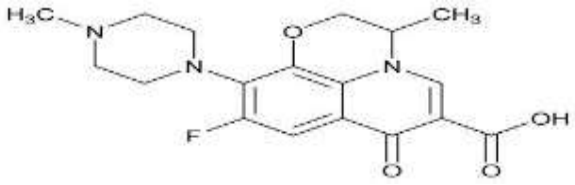

Figure 1: Structure of Ofloxacin

MATERIALS AND METHOD

Chemicals and reagents

Ofloxacin(99.4%) was obtained as gift sample from protect laboratories, Hyderabad, India. Pharmaceutical tablet formulation of OFLOX tab 200 mg purchased from local market. Urea(A.R Grade;Qualigens) and distilled water used for the study.

Instrumentation

Shimadzu UV -1800 double beam spectrophotometer with 1cm path length supported by shimadzu UV-probe software ,version 2.21 was used for spectral measurements with 10mm matched quartz cells . Shimadzu balance (BL-220H) was used for weighing.

Selection of solvent

8M urea solution was used as a solvent for developing spectral characterstics of a drug. The selection was made after assessing the solubility in different hydrotropic solvents like sodium acetate, sodoim benzoate, piperazine, sodium chloride, citric acid.Among these solvents ofloxacin was freely soluble (1 in 10 parts as per IP-2010) in 8 M urea and showed maximum drug stability.

Preparation of reagent solution

8M urea solution was prepared by 48.6gm of urea pure chemical was weighed and dissolved in 10 ml distilled water and the volume was made upto the mark with distilled water in 100 ml volumetric flask.

Preparation of standard stock solution

Working standard ofloxacin10 mg was weighed accurately and transferred to a 10 ml volumetric flask and dissolved in 1 ml of 8M urea solution. The flask was shaken and volume was made up to the mark withdistilled water to give a solution of 1000µg/ml. It was further diluted with distilled water to get the concentration of 100µg/ml. from this solution a series of aliquots were prepared for further method development.

Method A: Absorption maxima method

For the selection of analytical wavelength 10µg/ml solution of ofloxacin was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 nm to 400 nm. From the spectrum λ_{max} of ofloxacin, 288 nm was selected for the analysis. The calibration curve was prepared in concentration range of 3-15µg/ml at 288 nm.

Method B: Area under curve method

For the selection of analytical wavelength 10µg/ml solution of ofloxacin was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 nm to 400 nm. Area under curve (AUC) method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths 284-292 nm. Area calculation processing item calculates area bound by curve and horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration.

Method C:First order derivative spectroscopy

It involves the conversation of normal spectrum to its zero, first, second or higher derivative spectrum. In derivative spectrophotometry, spectra are obtained by plotting the first or a higher order derivative of absorbance with respect to wavelength as a function of wavelength. Often, these plots reveal spectral detail that is the lost in an ordinary spectrum. In addition, concentration measurements of an analyte in the presence of interference or of two or more analytes in a mixture can sometimes be made more easily accurately using derivative methods. In this method, 10µg/ml solution of ofloxacin was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200-400 nm. The absorption spectra thus obtained were derivitised from zero to second order. First order derivative spectra of drug showed a sharp peak at 316 nm, which was selected for its quantification.

Method validation

The method was validated according to ICH guidelines to study accuracy,linearity and precision.

Linearity

In order to find out linearity range of proposed UV-spectrophotometric method, studies were carried out by plotting absorbance of analyte against concentrations of the analyte.

A**ccuracy**

Accuracy is expressed as the closeness of the results from standard samples to that of the actual known amounts to determine the accuracy of the proposed method, recovery studies were carried out by adding different amounts (1.5µg, 3µg, 4.5µg) of bulk sample to the pre-analyzed formulation .the solutions were suitably diluted in the range and then each of the dilution was observed 6 times.

Precision

Precision is the level of repeatability of results as reported between samples analyzed on the same day (intra-day) and samples run on 3 different days (inter-day).to check the intra-day and inter-day variation of the method, solution containing 4.5 µg/ml ofloxacin were subjected to the proposed spectrophotometric method of analysis and the recoveries obtained were noted. the precision of proposed method i.e. the intra and inter-day variations in the absorbance of the drug solutions was calculated.

LOD

It is the lowest amount of analyte in a sample that can be detected but not necessarily quantities as an exact value under the stated, experimental conclusions. the detection limit is usually expressed as the concentration of analyte.

The standard deviation and response of the slope-

LOD=3.3 * standard deviation (σ)/ s

LOQ

The quantitation limit of an analytical procedure is the lowest amount of an analyte in a simple which can be quantitatively determined with suitable precision and accuracy.

The standard deviation and response of the slope-

LOQ=10* standard deviation (σ)/ s

Assay for estimation of ofloxacinin tablet formulation

For the estimation of ofloxacin in the commercial formulation, 20 tablets each containing 200 mg of ofloxacinwere weighed and average weight calculated. Triturate the tablets, for the analysis of drug, quantity of powder equivalent to 10 mg of ofloxacin was transferred to10 ml volumetric flask and dissolved in 8M Urea solution. It was filtered with whatmann filter paper no.41 and then volume made up to the mark with water to obtain a stock solution of 1000 µg /ml of ofloxacin, further dilutions of the stock solution were made in distilled water to get required concentration

RESULTS AND DISCUSSION

For quantitative estimation of ofloxacin in bulk and tablet dosage form three validated methods was proposed for method A, the absorbance maxima was found to be 288 nm (Figure-2&3), for method C λ_{max} at 316 nm was selected(Figure-6&7) and for method B area under curve in the range of 284-292 nm were selected for the analysis(Figure-4&5). The % assay by the three methods was found to be 100.8% in method A, 93.77% in method B and 98.0% in method C (table-4). No interference was observed from the pharmaceutical excipients. The % recovery obtained for absorption maxima, first order derivative spectroscopy and area under the curve was found to be in the range of 94.91%, 97.82%, 97.6%.(table-2), the proposed methods are very precise, the %RSD is less than 2 (table-3) and LOD&LOQ values of proposed methods are with in the limits.(table-1)Hence, the proposed methods were validated in terms of linarity, precision, and accuracy. The present work provides an accurate and sensitive method for the analysis of ofloxacin in bulk and tablet formulation.

Method A: Absorption maxima method

For the selection of analytical wavelength 10µg/ml solution of ofloxacin was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 nm to 400 nm. From the spectrum λ_{max} of ofloxacin, 288 nm was selected for the analysis. The calibration curve was prepared in concentration range of 3-15µg/ml at 288 nm. The calibration curve for

ofloxacin was plotted in the concentration v/s absorbance and regression equation was calculated.(Figure. 2&3)

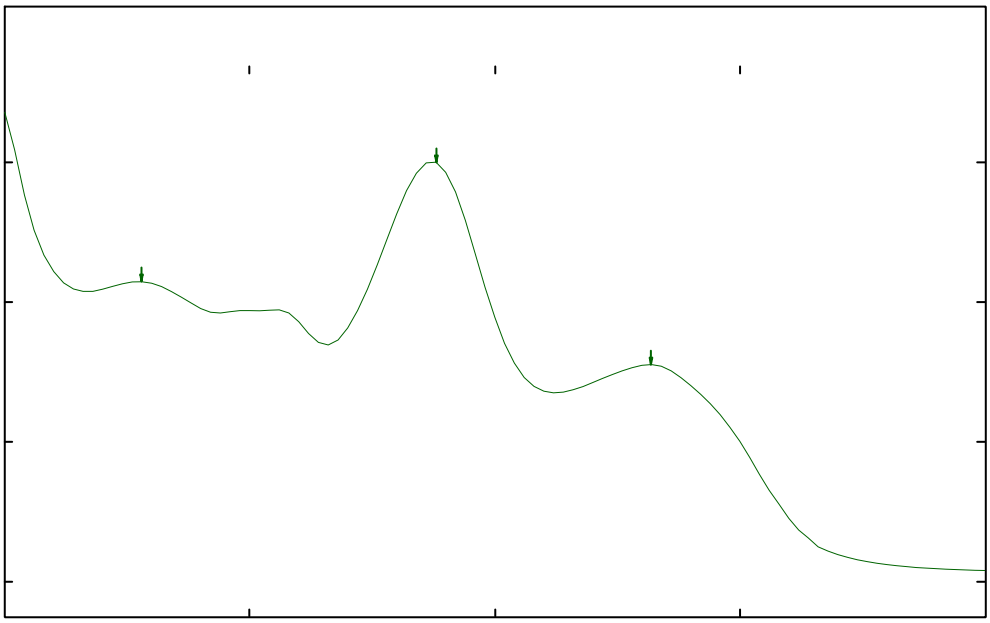

Figure 2: Absorption maxima spectrum of ofloxacin

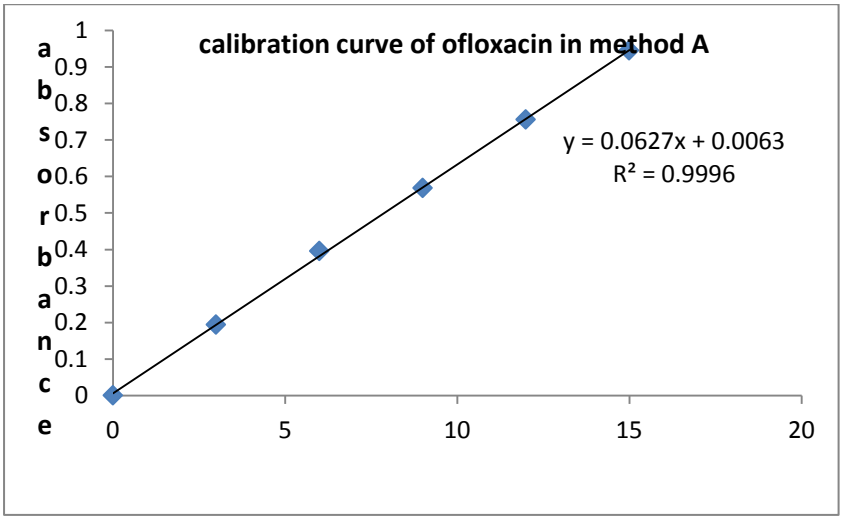

Figure 3: Calibration curve of ofloxacinin method A

Method B: Area under curve method

For the selection of analytical wavelength 10µg/ml solution of ofloxacin was prepared by appropriate dilution of standard stock solution and scanned in the spectrum mode from 200 nm to 400 nm. Area under curve (AUC) method involves the calculation of integrated value of absorbance with respect to the wavelength between two selected wavelengths 284-292 nm. Area calculation processing item calculates area bound by curve and horizontal axis. The horizontal axis is selected by entering the wavelength range over which the area has to be calculated. The

wavelength range is selected on the basis of repeated observations so as to get the linearity between area under curve and concentration. From this regression equation was calculated for the determination of amount of Ofloxacinin tablet formulation. (Figure.4 & 5)

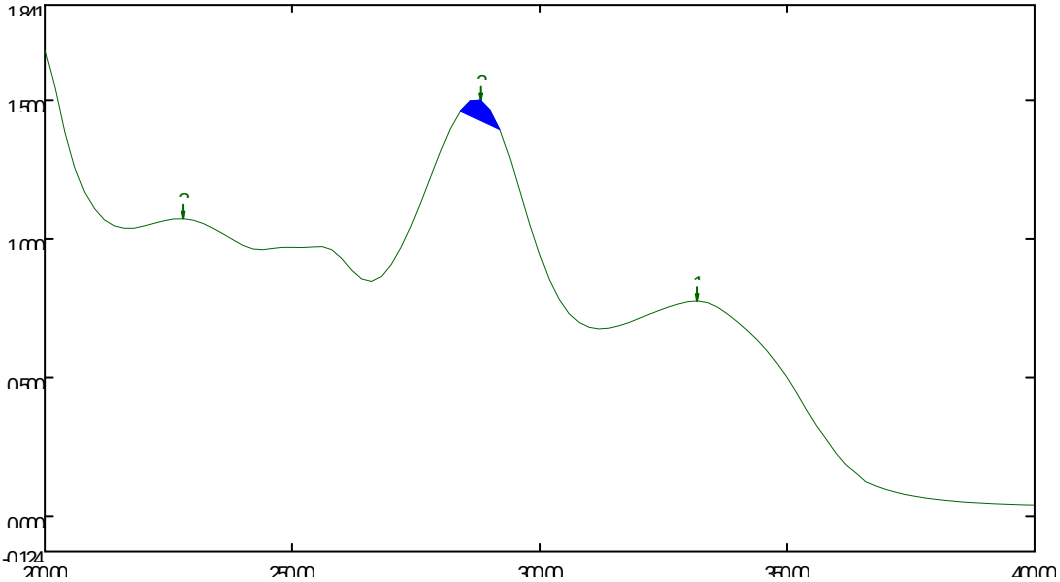

Figure.4: AUC spectrum of ofloxacin

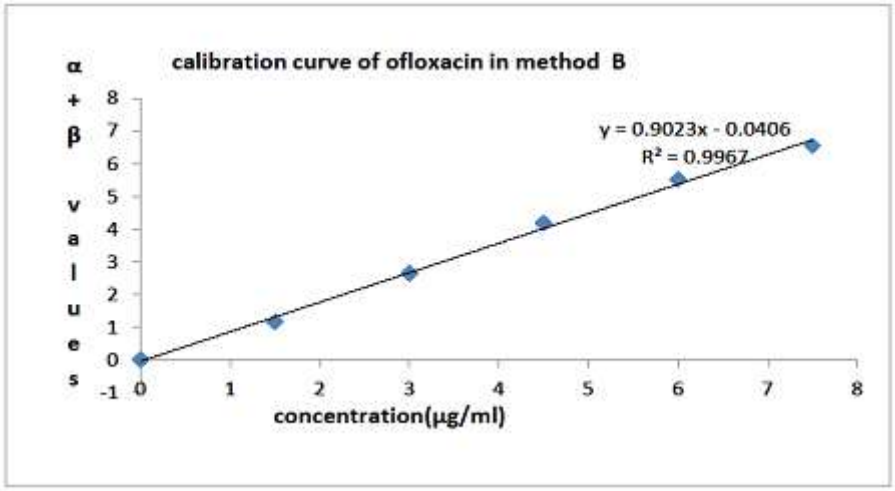

Figure. 5 calibration curve of ofloxacinin method B

Method C:First order derivative spectroscopy

It involves the conversation of normal spectrum to its zero, first, second or higher derivative spectrum. In derivative spectrophotometry, spectra are obtained by plotting the first or a higher order derivative of absorbance with respect to wavelength as a function of wavelength. Often, these plots reveal spectral detail that is the lost in an ordinary spectrum. In addition, concentration measurements of an analyte in the presence of interference or of two or more analytes in a mixture can sometimes be made more easily accurately using derivative methods. In this method, 10µg/ml solution of ofloxacin was prepared by appropriate dilution of standard stock solution and scanned

in the spectrum mode from 200-400 nm. The absorption spectra thus obtained were derivitised from zero to second order. First order derivative spectra of drug showed a sharp peak at 316 nm, which was selected for its quantification. The calibration curve for ofloxacin was plotted in the concentration range of 1.5-7.5 µg/ml at 316 nm. The concentration of drug present in the solution was determined against the calibration curve in quantization mode.(Figure. 6&7)

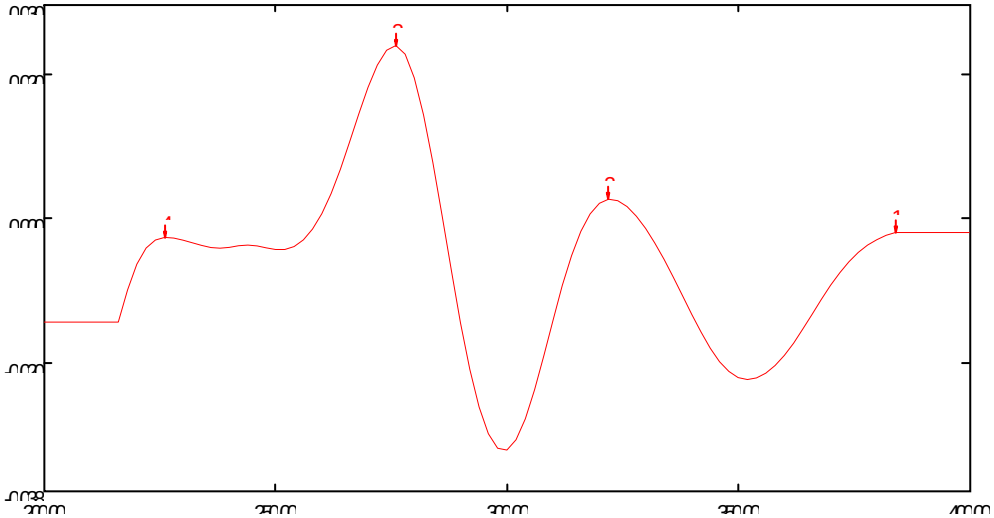

Figure.6 first order derivative spectrum of ofloxacin

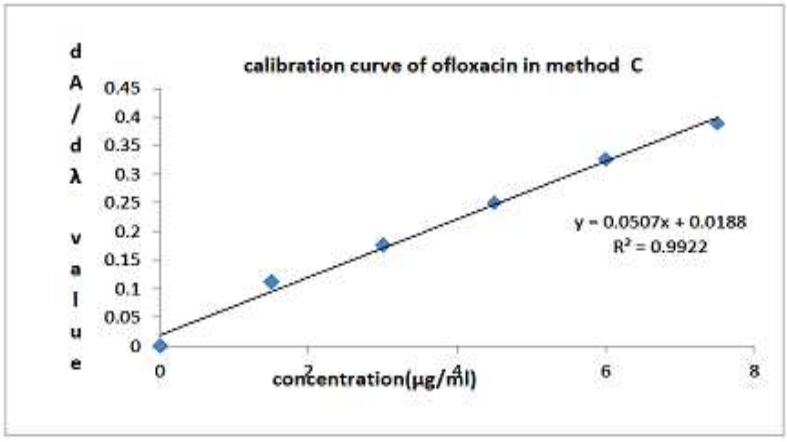

Figure. 7 Calibration curve of ofloxacinin method C

Method validation

The method was validated according to ICH guidelines to study accuracy,linearity and precision.

Linearity

A good linear relationship (r^2=0.999, 0.996 &0.992 for method A,B&C respectively) was observed between concentrations of ofloxacin and the corresponding absorbance. The regression of ofloxacin concentration over its absorbance was found to be y=0.062x+0.016, Y=0.902x-0.04&Y=0.050x+0.018for method A, B&C (where y is the absorbance and x is the concentration of ofloxacin).the slope, intercept and the correlation coefficient of the drug were shown in table.1

Table-1: Optical characteristics of the proposed methods

S.NO.	Parameter	Method A	Method B	Method C
1	Linearity(µg/ml)	3-15	1.5-7.5	1.5-7.5
2	Linearity eqution	y=0.062X+0.006	Y=0.902x-0.04	Y=0.050x+0.018
3	Slope±SD	0.062±0.026	0.902±0.035	0.050±0.028
4	Intercept ±SD	0.006±0.046	0.04±0.069	0.019±0.096
5	Correlation coefficient	0.999	0.996	0.992
6	LOD	150ng	160ng	145ng
7	LOQ	450ng	480ng	435ng

Accuracy

The % recoveries of the drug was found to be 94.91, 97.6 &97.82% in method A, B & C respectively. The results were shown in the table.2

Table-2: Recovery studies of proposed methods

Method	Level of recovery	Pre analyzed conc(µg/ml)	Amount added(µg/ml)	Amount found(µg/ml)(n=6)	%Recovery	%RSD
Method A	50	3	1.5	4.22	93.77	0.024
	100	3	3	5.62	93.66	
	150	3	4.5	7.3	97.3	
Method B	50	3	1.5	4.34	96.44	0.013
	100	3	3	5.75	95.83	
	150	3	4.5	7.54	100.53	
Method C	50	3	1.5	4.53	100.66	0.041
	100	3	3	5.65	94.16	
	150	3	4.5	7.40	98.66	

Precision

In terms of % RSD and the results were presented in the table.3 stastical revolution revealed that relative standard deviation of drugs at different concentration levels for 6 times was less than 2.0 (intraday-0.177inter day-0.147).

Table-3 Precision studies of proposed methods

Intra day				Inter day		
Method	**Concentration (µg/ml)**	**Mean ±SD**	**%RSD**	**Concentration (µg/ml)**	**Mean ±SD**	**%RSD**
A	4.5	4.31±0.0046	0.137	4.5	4.44±0.0065	0.135
B	4.5	4.29±0.0072	0.165	4.5	4.37±0.0045	0.164
C	4.5	4.34±0.0015	0.231	4.5	4.29±0.0076	0.144

Assay for estimation of ofloxacinin tablet formulation

In method A the concentration of ofloxacin was determined by measuring absorbance of sample solution at 288 nm .in method B, the concentration of ofloxacin was determined by measuring

absorbance of sample solution in wavelength range of 284-292 nm. In method C, first order derivative spectroscopy the concentration of ofloxacinwas determined by measuring amplitude difference at λ_{max} 316 nm. Result of tablet analysis are shown in table no. the assay procedure was repeated 6 times (n=6) (table.4)

Table 4: Results of marketed formulation analysis

Proposed methods	Label claim(mg)	Test conc(µg/ml)	Amount found (µg/ml)	%Assay	%RSD
A	200mg	6	6.04	100.8	0.325
B	200mg	4.5	4.22	93.77	0.656
C	200mg	4.5	4.41	98.00	0.745

CONCLUSION

The three spectrophotometric methods were developed and validated as per ICH guidelines. The standard deviation and %RSD calculated for the methods are within the limits, indicating high degree of precision of the methods. The results of the recovery studies performed indicate the methods to be accurate. Hence it can be conducted that the developed spectrophotometric methods are accurate, precise and can be employed successfully for the estimation of ofloxacin bulk and formulation. The proposed methods were found to be simple, economical, eco-friendly, rapid, precise and accurate for the determination of ofloxacin in tablet dosage form. There is good scope for other poorly water soluble drugs which may be tried to get solubilized in 8M ureasolution (as hydrotropic agent) to carry out their spectrophotometer analysis excluding the use of costlier and unsafe organic solvents. Thus, it can be easily and conveniently adopted for routine quality control analysis.

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